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Significant role for lifetime cigarette smoking in worsening bladder cancer and upper tract urothelial carcinoma prognosis: a meta-analysis

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ABSTRACT

Purpose

Although cigarette smoking is a well-established risk factor for urothelial cancer (UC), its role in UC prognosis is still undetermined. This meta-analysis aimed to quantify the role of lifetime smoking history on bladder cancer recurrence, progression and survival, by pooling available data on non-muscle-invasive bladder cancer (NMIBC), muscle-invasive bladder cancer (MIBC) and upper tract urothelial carcinoma (UTUC).

Materials and methods

A total of 24 studies, comprising data from 13,114 BC and 2,259 UTUC patients, were included in this meta-analysis. Publication bias was addressed through Eggers test and the heterogeneity between studies was assessed by the I^2 test statistic and subgroup analyses.

Results

Current smokers at diagnosis are at an increased risk of developing local recurrences in NMIBC (HR=1.27, 95% C.I. = 1.09-1.46) and smoking MIBC patients are at an increased risk of dying due to BC (HR=1.23, 95% C.I. = 1.02-1.44). In the UTUC population smokers have both an increased risk of recurrence in the operative bed (HR=1.57, 95%C.I. = 1.19-1.95) and of dying due to UTUC (HR=1.53, 95%C.I. = 1.13-1.92). We did not identify significant heterogeneity between included studies.

Conclusions

The body of evidence is limited due to the absence of prospective studies. However, the results from this meta-analysis unambiguously support the hypothesis that lifetime cigarette smokers are at an increased risk of developing a more malignant type of urothelial carcinoma associated with worse prognosis.

Keywords: bladder cancer; upper tract urothelial carcinoma; cigarette smoking; prognosis; meta-analysis

1. PURPOSE

Smoking is a major risk factor for the development of urothelial cancer as has been shown repeatedly in several case-control and cohort studies (1). However, there has been little research into the influence of smoking status at diagnosis, and continuation of smoking after diagnosis, on urothelial cancer recurrence, progression and survival. Several reviews have highlighted the potential effect that smoking status might have on disease recurrence in non-muscle-invasive bladder cancer (NMIBC) and possibly progression to muscle-invasive bladder cancer (MIBC) (2-4) as well as worse upper tract urothelial carcinoma (UTUC) prognosis (2, 5).

Lifetime smoking history seems to be associated with increased tumour size and aggressiveness of disease at first diagnosis (4, 6). However, it remains unclear whether this is also reflected in different clinical outcomes from urothelial carcinoma (UC), a definition which encompasses both BC and UTUC, for patients who are smokers compared to never smokers. It is suggested that current smokers at diagnosis have higher NMIBC local recurrence rates compared to never smokers (2, 3). However, this association or the potentially significant association with disease progression has never been previously summarised in a meta-analysis.

This study aimed to quantify the role of lifetime smoking history on UC recurrence, progression and survival, by meta-analysis of available data on NMIBC, MIBC and UTUC. By quantifying the associations between lifetime smoking status and UC prognosis, this study will shed more light on whether smoking status should be incorporated in prognostication of BC and UTUC in clinical as well as non-clinical settings.

2. MATERIALS AND METHODS

2.1. Search strategy

Medline, Embase, Web of Science and Scopus were used to search for studies on cigarette smoking and bladder cancer recurrence, progression and bladder-cancer related mortality. The search was restricted to only include studies involving humans and there was no restriction with regard to publication date. Search terms included the (MeSH) terms “urinary bladder neoplasms”, “neoplasm recurrence” “survival”, “smoking” and “epidemiologic studies” which resulted in 1,528 hits on possible articles to include after removal of duplicates. Studies were excluded when no hazard ratio (HR) for either recurrence-free, progression-free or disease-free survival for smoking status (current and former versus never smokers) was given. Disease recurrence had to be specified as local recurrence for NMIBC and MIBC and as recurrence in the operative bed of the tumour for UTUC. Furthermore, only studies which regarded never smokers as the reference category for calculation of HRs were included. Moreover, only studies which provided information or stratified for received therapy were included to avoid misinterpretation due to the effects of different therapies.

2.2. Data collection

To assess the risk of bias and identify possible sources of heterogeneity within individual studies selected for full text evaluation the Newcastle-Ottowa assessment scale (NOS) for cohort studies was applied (7) by two of the authors (FvO and SJ). Several variables were extracted from included articles including year of publication, geographic area, disease type (NMIBC, MIBC or UTUC), cigarette smoking assessment (questionnaire, interview or patient records), factors adjusted for and median length of follow-up time. HRs were obtained directly from included articles and were divided in recurrence-free (RFS), progression-free

(PFS) and disease-specific (DSS) survival HRs. When bladders were resected in MIBC populations, recurrence-free survival was specified by loco-regional recurrence; recurrence at the margins of the surgical field or in the lymph nodes.

2.3. Statistical analysis

HRs were pooled comparing RFS, PFS and DSS for current and former smokers to never smokers in NMIBC, MIBC and UTUC patients. Because HRs could differ between studies, a random effects model was used to model potential heterogeneity. The I^2 test statistic was used to estimate between-study variance. Furthermore, meta-regression analysis was performed to estimate the effect of possible sources of heterogeneity (smoking assessment, factors adjusted for, length of follow-up) on different outcome measures. Publication bias was explored by funnel plots and Egger's test for small study effects for the different analyses performed (8). Unfortunately, there was insufficient data to also stratify for gender. Subgroup analyses were performed comparing pooled HRs across geographic area, length of follow-up quartile, study design and smoking assessment where possible. Stata statistical software was used for all analyses (version 13; Stata Corp., College Station, TX).

3. RESULTS

3.1. Study characteristics

In total, 24 studies were included in this meta-analysis; 11 for NMIBC (9-19) including 7,210 cases, 7 for MIBC (20-26) investigating 2,259 cases, and 6 for UTUC (27-32) encompassing 5,904 cases (Table 1). During full-text evaluation 5 studies were excluded because of a lack of data in the study report (33-37). Furthermore, 9 articles were excluded during data extraction because no HR for smoking status was given (38-42) or because the reference category was not comprised of never smokers (43-46) (Figure 1). All included studies were patient cohort studies, except for two epirubicin trials in NMIBC (10, 13). The majority of studies which included HR estimates regarding smoking and UC prognosis were published after 2007. Smoking status was assessed either through questionnaires (9, 10, 12-15, 18-20, 22, 25, 27-32), patient records (11, 21, 23, 24, 26) or an interview (16, 17). All included study populations for NMIBC had undergone full transurethral resection of a bladder tumour (TURBT) with some recorded to have received Bacillus Calmette–Guérin (BCG) treatment (11, 16). For MIBC, only studies in which patient populations had undergone radical cystectomy (complete removal of the bladder) could be included, with some populations receiving (neo)adjuvant chemotherapy (20, 23, 24, 26). Although radiation therapy is becoming more common as an alternative to radical cystectomy (47), no studies investigating associations between smoking and outcomes were found in radiation therapy patient populations. In included UTUC study populations all patients underwent nephroureterectomy. (INSERT TABLE 1 & FIGURE 1 HERE)

3.2. Risk estimates for smoking status in NMIBC

Figure 2 summarizes all obtained risk estimates for smoking and RFS and PFS for NMIBC and shows the individual study results in forest plots. The pooled HR of 1.27 (95% C.I.=1.09-

1.46) shows that current smokers at diagnosis are at a slightly increased risk of developing local recurrences compared to non-smoking NMIBC patients. A mitigated effect is seen in former smokers compared to non-smokers where the pooled HR is 1.13 (95% C.I.=1.00-1.25) based on 5,382 NMIBC cases. Similar (but not statistically significant) pooled HRs for PFS were obtained for both smokers (HR=1.21, 95% C.I.=0.81-1.61) and former smokers (HR=1.13, 95% C.I.=0.81-1.45). With regard to DSS, only two studies investigating 925 NIMBC cases could be pooled and indicated no effect of being a current smoker at baseline (HR=1.01 (95% C.I.=0.93-1.10)). (INSERT FIGURE 2 HERE)

3.3. Risk estimates for smoking status in MIBC

Obtained pooled estimates for smoking status in MIBC are summarized and depicted in forest plots in Figure 3. With regard to RFS only one of the four included studies reported a significantly increased risk of loco-regional recurrence in MIBC for current smokers at diagnosis (21), and two of three included studies showed an increased risk of local recurrence for former smokers (20, 21). Pooling these studies resulted in a pooled HR for recurrence of 1.09 (95% C.I.=0.78-1.40) for current smokers and a HR of 1.17 (95% C.I.=0.82-1.52) for former smokers compared to non-smokers at diagnosis. For the relation between smoking status at baseline and DSS more studies were identified in the literature. Current smokers at diagnosis seem to be at a higher risk of dying due to BC compared to non-smokers (HR=1.23, 95% C.I. =1.02-1.44), while a similar effect was observed for former smokers (HR=1.26, 95% C.I. =0.98-1.54). The HR for current smokers did not seem to differ between studies with different follow-up times. (INSERT FIGURE 3 HERE)

3.4. Risk estimates for smoking status in UTUC

No published studies on PFS for smokers in UTUC patient populations could be identified. Current smokers at diagnosis were at a significantly higher risk of both developing

recurrences in the operative bed (HR=1.57, 95%C.I. =1.19-1.95) and dying due to UC (HR=1.53, 95%C.I. =1.13-1.92). For former smokers this effect was mitigated, both for RFS (HR=1.31, 95% C.I.=0.85-1.78) and DSS (HR=1.20, 95% C.I. =0.80-1.61) (Figure 4). (INSERT FIGURE 4 HERE) Although the evidence was too sparse or limited to pool for NMIBC and MIBC studies for UTUC there were two similar studies (28, 29) investigating smoking duration and intensity before diagnosis on RFS. Results from these studies indicate a dose-response relationship between both smoking duration and intensity and RFS in UTUC patients (Online supplemental materials – Table 1).

3.5. Publication bias and heterogeneity between studies

Both statistically and visually (as judged from several funnel plots), the amount of bias between studies seemed negligible within all three different disease categories. However, a statistically significant small study effect (p=0.041) was observed for the NMIBC studies investigating RFS, indicating that there were no (small) studies published which observed a protective effect of smoking. Meta-regression analyses showed that no significant heterogeneity was present due to mode of smoking assessment, number of adjusted factors (mostly multivariable) or months of median follow-up, and I^2 test statistics were all under 30% except for comparing the five studies investigating RFS in UTUC (78%).

3.6. Sensitivity analyses

Subgroup analyses in studies investigating RFS in NMIBC patients and DSS in MIBC showed no significant heterogeneity between geographic areas or follow-up quartile and study design or smoking assessment respectively between studies (Figure 5). Current smokers from Asian populations (16-18) seemed to be at a higher risk of developing local recurrences compared to American, European and worldwide populations, although these differences were not statistically significant (p=0.452, p=0.447 and p=0.452 respectively). Within all

included UTUC studies research methods were homogenous thus results could not be stratified for sensitivity analysis. Additional inverse variance weighted regression analyses showed no significant change in observed HRs with increasing median follow-up time continuously. (INSERT FIGURE 5 HERE).

4. DISCUSSION

This meta-analysis indicates a role for lifetime smoking behaviour in BC and UTUC prognosis, showing an increased risk for disease recurrence and increased risk of death for current smokers at different stages of BC and UTUC.

4.1. Significantly higher local recurrence rates for smokers at diagnosis in NMIBC

Previous reviews have already suggested that current smokers at diagnosis are at an increased risk of developing local recurrences compared to non-smokers in NMIBC patient populations (2-4); however, this is the first study to quantify these risks. We observed an increased risk of developing local recurrences for current smokers compared to never smokers. Former smokers had a lower (but still increased) risk of developing local recurrences. These results are in-line with other studies indicating a potentially more malignant disease type for smokers at diagnosis (4, 6). The associations between smoking status at diagnosis and PFS were comparable to the RFS estimates, although not statistically significant.

In NMIBC, smoking appears to promote recurrences but it is not associated with progression/death. Mechanistically, there is thus likely to be an influence of smoking on the hallmark capabilities of BC such as self-sufficiency in growth signals and delimiting replicative potential (48, 49). Although evidence on the effects of smoking with regard to BC prognosis is limited, including smoking status in panels of molecular markers (including e.g. p53, cyclooxygenase and vascular endothelial growth factor) has shown to improve prognostication in several studies (45, 50).

4.2. More deaths due to BC in smokers at diagnosis compared to never smokers in MIBC

Compared to NMIBC there were considerably fewer studies investigating the role of smoking in MIBC prognosis, although one other meta-analysis also showed significantly increased BC mortality for current smokers compared to never smokers (RR=1.89 95% C.I.= 1.29-2.78) not regarding stage of disease (51). We did not demonstrate a relationship between smoking and RFS in MIBC patients; however, both current and former smokers at diagnosis are at a higher risk of dying due to BC compared to never smokers. There were also noticeably fewer studies investigating RFS compared to DSS in MIBC patient populations, emphasizing the need for more studies investigating RFS also in MIBC patients. Although there have not been specific studies investigating this association biologically or mechanistically, it is possible that the higher mortality due to bladder cancer for smokers can be attributed to a more malignant subtype of BC at diagnosis, as suggested by other authors (4, 6).

With regard to smoking and survival in MIBC, there are several malignant processes in which smoking is suspected to play a role and which might explain the observed association with poor survival. Smokers have been shown to have a significantly higher expression of *Twist*, a transcription factor regulating epithelial-mesenchymal transition which is an important event in tumor invasion (52). Also, positive expression of heme-oxygenase-1 (HO-1) may be dependent on smoking intensity as measured at diagnosis (53). Moreover, it is probable that chronic exposure to cigarette smoke induces reduced sensitivity to cisplatin treatment, a well-known phenomenon in the management of MIBC (54).

4.3. Significant associations between smoking and both RFS and DSS in UTUC patients

Interestingly, current smokers at diagnosis in the UTUC population were approximately at a 50% increased risk of both RFS and DSS compared to never smokers. Although the number of events was the smallest from the three meta-analyses performed, significant associations were observed. This indicates that also for UTUC patients, the risk of developing disease

recurrences in the operative bed and dying due to UTUC is increased for smokers. Although similar associations are observed and many disease characteristics are shared, UTUC is considered to be a different disease than BC because there are a number of anatomical, biological and molecular-genetic differences (55). For example, microsatellite instability (MSI) and hypermethylation are more often observed in UTUC than in BC, which might lead to somatic inactivation of DNA mismatch repair genes representing a different pathway of initiating events (56). Furthermore, the proportion of tumours that are invasive at diagnosis is about 50% for UTUC (57) whereas for BC these numbers are lower at approximately 20-30% (58), possibly explaining why the observed associations with RFS and DSS are stronger compared to those found in NMIBC and MIBC.

4.4. Limitations

Important limitations of this study were the lack of prospective data on smoking behaviour after diagnosis, insufficient data to pool for relevant molecular subtypes of NMIBC and the lack of more detailed smoking behaviour (such as duration and intensity) before diagnosis in survival analyses. Even though current smokers at diagnosis could have quit smoking in the period prior to recurrence, dose-response meta-analyses show that smoking cessation only results in risk reduction at least 15 years before diagnosis, indicating a long latency effect of cigarette smoke in determining UC risk *(unpublished results van Osch et al., manuscript currently under review).* When considering recurrence as an incident event which usually occurs within 15 years of diagnosis, looking at lifetime smoking status at diagnosis could be a good proxy measure (with relatively high consistency between included studies for the effect of smoking on the development of recurrence). The same arguments hold for progression and UC mortality events. However, since UC is extremely heterogeneous biologically (59), studies prospectively investigating smoking within strata of molecular subtype and stage will provide a more thorough quantification of the effect of cigarette smoking in UC prognosis

(and could additionally correct for smoking cessation after diagnosis in analyses to further enhance the biological plausibility of these results). Furthermore, such studies will be better able to address the question whether smoking status should be considered in predictive nomograms (e.g. EORTC or CUETO) to improve decision-making in BC treatment.

5. CONCLUSIONS

This meta-analysis supports the hypothesis that lifetime cigarette smokers are at an increased risk of developing a more malignant type of UC. Smoking NMIBC and MIBC patients at diagnosis are at an approximately 25% increased risk of developing local recurrences and dying due to BC respectively. Furthermore, smoking UTUC patients are at an approximately 55% increased risk of both developing recurrences in the operative bed of the tumour and dying due to UTUC compared to never smokers. These results indicate a significant role for lifetime smoking status in both BC and UTUC prognosis.

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APPENDIX A – Table & Figure legends

Table 1. Study characteristics of included studies investigating smoking in bladder cancer prognosis, ordered by year of publication

Figure 1. Flowchart of study selection and reasons for exclusion

Figure 2. Pooled risk estimates for recurrence-free survival (RFS) and progression-free survival (PFS) in non-muscle invasive bladder cancer (NMIBC) including forest plots depicting individual study results

Figure 3. Pooled risk estimates for recurrence-free survival (RFS) and disease-specific survival (DSS) in muscle invasive bladder cancer (MIBC) including forest plots depicting individual study results

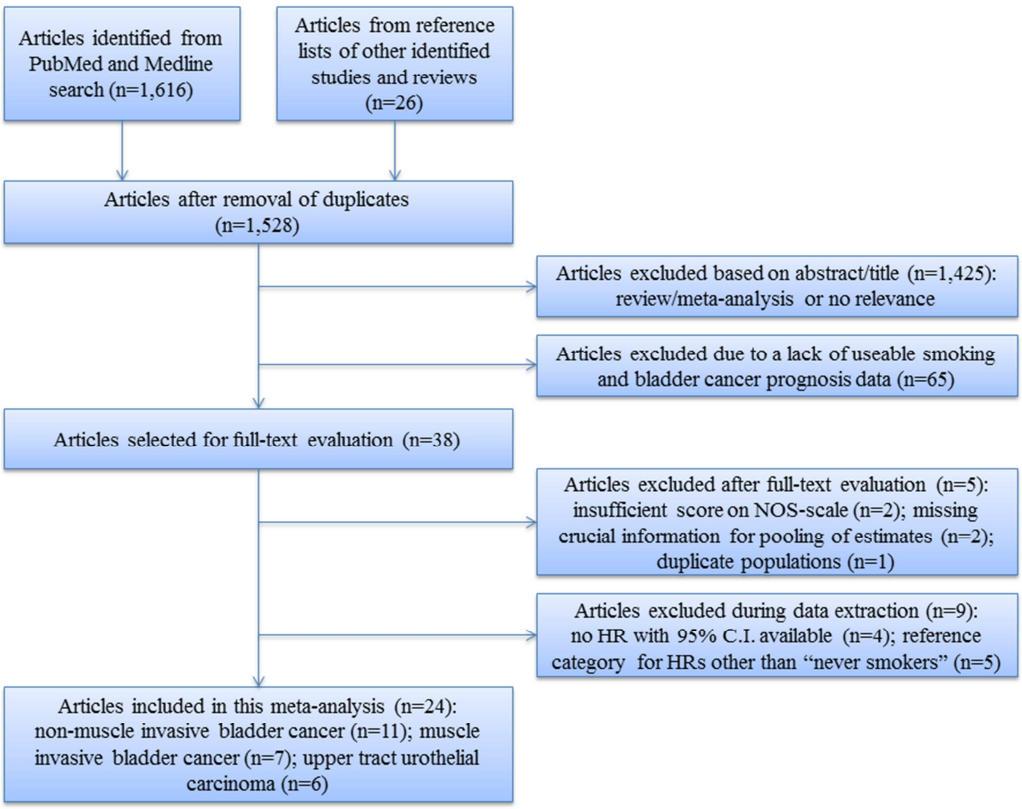
Figure 4. Pooled risk estimates for recurrence-free survival (RFS) and disease-specific survival (PFS) in upper tract urothelial carcinoma (UTUC) including forest plots depicting individual study results

Figure 5. Forest plot depicting summary hazard ratios (HR) for recurrence-free survival (RFS) comparing current smokers versus non-smokers in NMIBC (panel a)and disease-free survival (DSS) in MIBC (panel b), by geographic area and time of follow-up quartile and study design and smoking assessment respectively. The dashed line represents no effect and the solid line stands for the overall pooled HR.

Table 1. Study characteristics of included studies investigating smoking in bladder cancer prognosis, ordered by year of publication

Reference	First Author	Year	Country	Disease type	Number of cases	Type of study	Length of follow-up (median in months)	Cigarette smoking assessment
(9)	Grotenhuis	2014	The Netherlands	NMIBC	963	cohort	60	Questionnaire
(10)	Wyszynski	2014	USA	NMIBC	716	cohort	72	Questionnaire
(11)	Rink	2013	Europe & USA	NMIBC	2,043	cohort	49	Questionnaire
(12)	Serretta	2013	Italy	NMIBC	395	trial	48	Questionnaire
(13)	Lammers	2011	The Netherlands	NMIBC	278	trial	30	Questionnaire
(14)	Segal	2011	Canada	NMIBC	718	cohort	36	Questionnaire
(15)	Sfakianos	2011	USA	NMIBC	623	cohort	81	Patient records
(16)	Gangawar	2010	India	NMIBC	135	cohort	14	Interview
(17)	Chen	2008	Taiwan	NMIBC	413	cohort	36	Interview
(18)	Chen	2007	Taiwan	NMIBC	256	cohort	38	Questionnaire
(19)	Allard	1995	Canada	NMIBC	368	cohort	24	Questionnaire
(20)	Kim	2014	USA	MIBC	139	cohort	43	Questionnaire
(21)	da Silva	2013	Europe, Brazil & USA	MIBC	642	cohort	34	Patient records
(22)	Lee	2012	Korea	MIBC	602	cohort	56	Questionnaire
(23)	Bostrom	2011	USA	MIBC	564	cohort	50*	Patient records
(24)	Boorjian	2011	Canada & Finland	MIBC	1,506	cohort	43	Patient records
(25)	Yafi	2010	Canada	MIBC	2,287	cohort	35	Patient records
(26)	Batty	2008	UK	MIBC	164	cohort	-	Questionnaire
(27)	Kluth	2014	Worldwide	UTUC	242	cohort	33	Questionnaire
(28)	Xylinas	2014	Worldwide	UTUC	519	cohort	37	Questionnaire
(29)	Rink	2013	Turkey	UTUC	864	cohort	50	Questionnaire
(30)	Gunay	2013	Japan	UTUC	101	cohort	56*	Questionnaire
(31)	Hagiwara	2013	Europe & USA	UTUC	245	cohort	51	Questionnaire
(32)	Ehdaie	2012	USA	UTUC	288	cohort	50	Questionnaire

* mean length of follow-up in months instead of median length of follow-up



NMIBC	RFS				PFS			
Determinants	No. of studies	No. of events	HR	95% CI	No. of studies	No. of events	HR	95% CI
Smoking status								
Non-smoker			1.00	Reference			1.00	Reference
Former smoker	7[A]	1903	1.13	1.00-1.25	4[C]	978	1.13	0.81-1.45
Current smoker	10[B]	1723	1.27	1.09-1.46	6[D]	617	1.21	0.81-1.61

RFS=recurrence free survival, PFS=progression free survival, HR=hazard ratio, CI=confidence interval

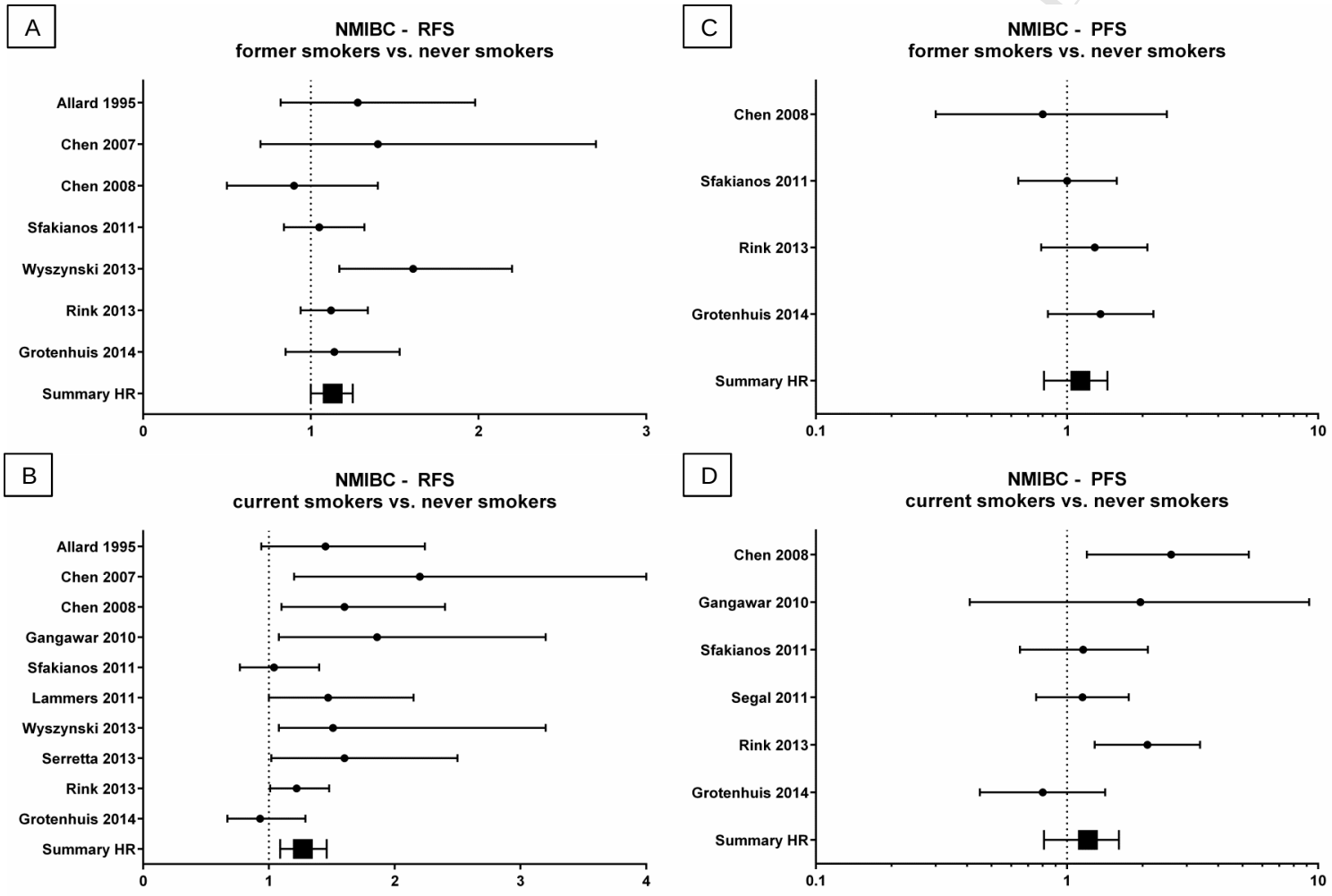


Figure 2. Pooled risk estimates for recurrence-free survival (RFS) and progression-free survival (PFS) in non-muscle invasive bladder cancer (NMIBC) including forest plots depicting individual study results

MIBC	RFS				DSS			
Determinants	No. of studies	No. of events	HR	95% CI	No. of studies	No. of events	HR	95% CI
Smoking status								
Non-smoker			1.00	Reference			1.00	Reference
Former smoker	3[A]	360	1.17	0.82-1.52	3[C]	402	1.26	0.98-1.54
Current smoker	4[B]	1206	1.09	0.78-1.40	6[D]	760	1.23	1.02-1.44

RFS=recurrence free survival, DSS=disease-specific survival, HR=hazard ratio, CI=confidence interval

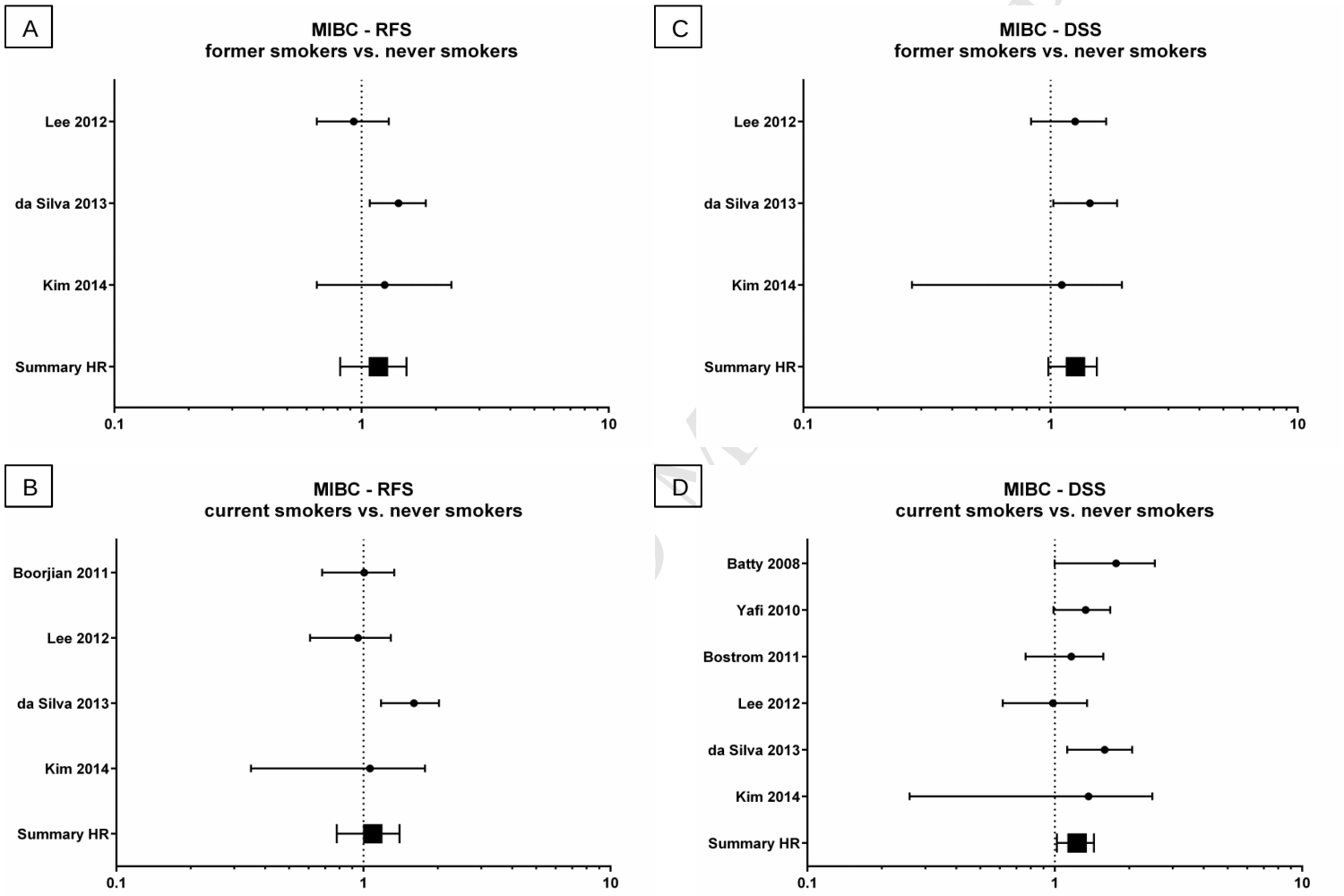


Figure 3. Pooled risk estimates for recurrence-free survival (RFS) and disease-specific survival (DSS) in muscle invasive bladder cancer (MIBC) including forest plots depicting individual study results

UTUC	RFS				DSS			
Determinants	No. of studies	No. of events	HR	95% CI	No. of studies	No. of events	HR	95% CI
Smoking status								
Non-smoker			1.00	Reference			1.00	Reference
Former smoker	4	415	1.31	0.85-1.78	2	331	1.20	0.80-1.61
Current smoker	5	482	1.57	1.19-1.95	2	330	1.53	1.13-1.92

RFS=recurrence free survival, DSS=disease-specific survival, HR=hazard ratio, CI=confidence interval

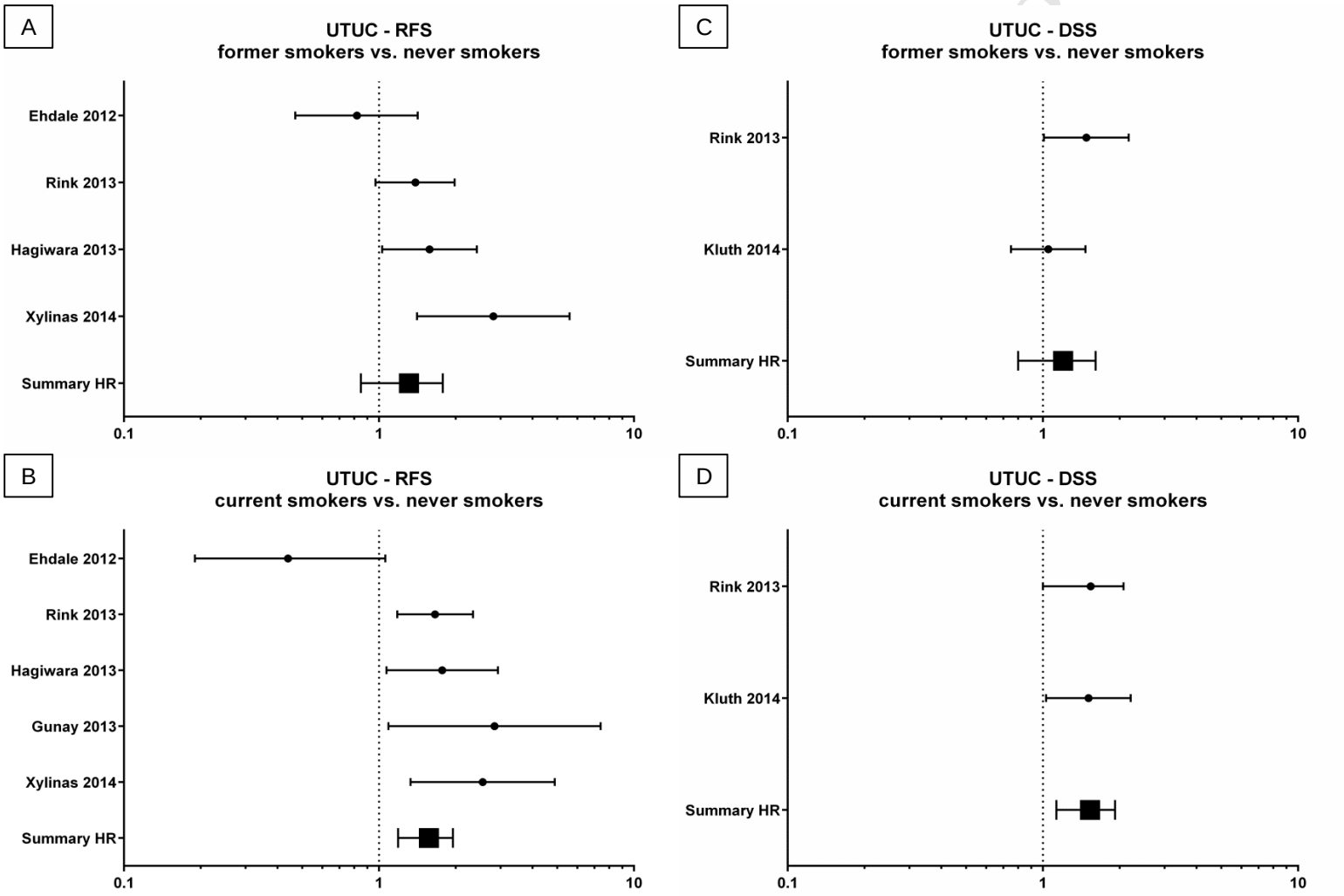
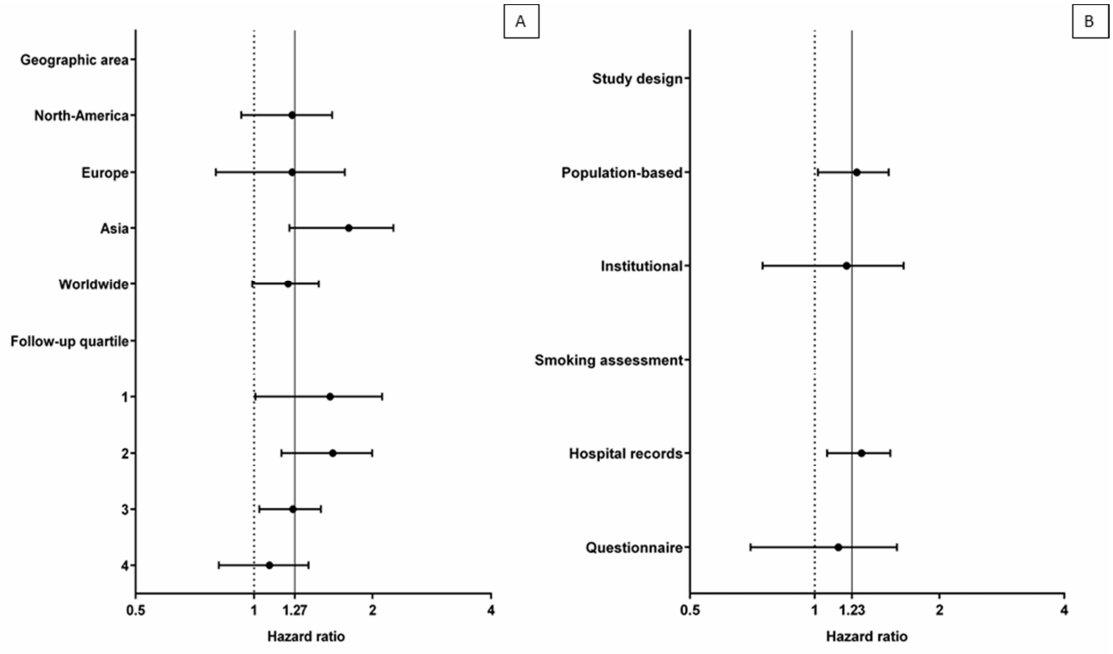


Figure 4. Pooled risk estimates for recurrence-free survival (RFS) and disease-specific survival (PFS) in upper tract urothelial carcinoma (UTUC) including forest plots depicting individual study results



Online Supplemental Table 1. Pooled hazard ratios for recurrence-free survival (RFS) for smoking duration and smoking intensity from two multicentre UTUC patient cohorts.

		RFS, UTUC		
		n	**HR**	**95% C.I.**
Duration (years)				
	0		1.00	ref
	<20	2	1.42	0.94-1.91
	≥20	2	1.58	1.16-2.00
Intensity (cigarettes per day)				
	0		1.00	ref
	<20	2	1.49	1.08-1.91
	≥20	2	1.64	1.15-2.12

Key of definitions for abbreviations:

BC = bladder cancer
C.I. = confidence interval
CUETO = Spanish Urological Club for Oncological Treatment scoring system
DSS = disease-specific survival
EORTC = European Organisation for Research and Treatment of Cancer scoring system
HR = hazard ratio
MIBC = muscle-invasive bladder cancer
NMIBC = non-muscle-invasive bladder cancer
NOS = Newcastle-Ottowa assessment scale
PFS = progression-free survival
RFS = recurrence-free survival
UTUC = upper tract urothelial carcinoma
UC = urothelial cancer